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Consumer Product Safety Commission

Environmental Protection Agency

Department of Health, Education, and Welfare

Food and Drug Administration

Department of Agriculture

Food Safety and Quality Service

Scientific Bases for Identification of Potential Carcinogens and Estimation of Risks

Requests for Comments on Reports

CONSUMER PRODUCT SAFETY COMMISSION

ENVIRONMENTAL PROTECTION AGENCY

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

DEPARTMENT OF AGRICULTURE
Food Safety and Quality Service

Scientific Bases for Identification of Potential Carcinogens and Estimation of Risks; Request for Comments on Report

AGENCIES: Consumer Product Safety Commission (CPSC); Environmental Protection Agency (EPA); Food and Drug Administration, Department of Health, Education and Welfare (FDA); Food Safety and Quality Service, Department of Agriculture (FSQS)

ACTION: Request for public comment on scientific report.

SUMMARY: This notice publishes and requests comment on a scientific report entitled: "Scientific Bases for Identification of Potential Carcinogens and Estimation of Risks." The report was written by the Work Group on Risk Assessment of the Interagency Regulatory Liaison Group (IRLG) with the assistance of senior scientists at the National Cancer Institute (NCI) and the National Institute of Environmental Health Sciences (NIEHS). The report represents the best judgments of these scientists and those of the four agencies (CPSC, EPA, FDA, and the Occupational Safety and Health Administration (OSHA)) comprising the IRLG at the time the report was written on the scientific concepts and methods currently in use to identify and evaluate substances that may pose a risk of cancer to humans. The FSQS has since joined IRLG. Scientists at FSQS have reviewed the report and concur. The report is being published by CPSC, EPA, FDA, and FSQS for comment in order to give interested persons an opportunity to express their views on the validity and appropriateness of the concepts and methods described for identifying and evaluating carcinogens. After reviewing the comments received, the four agencies anticipate publishing a statement giving notice of whatever revisions to the document are appropriate, if any.

DATE: Written comments on the report should be submitted by September 30, 1979.

ADDRESS: Comments should be sent to IRLG, Room 500, 1111 18th Street, N.W., Washington, D.C. 20207.

FOR FURTHER INFORMATION CONTACT:
Susan Guenette at (202–634–4350).

SUPPLEMENTARY INFORMATION:

Background

In August, 1977, The Consumer Product Safety Commission, the Environmental Protection Agency, the Food and Drug Administration of the Department of Health, Education, and Welfare, and the Occupational Safety and Health Administration of the Department of Labor agreed to work together as the Interagency Regulatory Liaison Group (IRLG) to improve protection of the public health and the environment through sharing of information, avoiding duplication of effort, and developing consistent regulatory policy.

On October 11, 1977, the IRLG published in the **Federal Register** an Interagency Agreement relating to the Regulation of Toxic and Hazardous Substances (42 FR 54856). To implement this agreement, the IRLG established work groups to develop common, consistent, or compatible practices in areas of activities common to the four activities, including risk assessments. Provisional work plans were published for the work groups in the **Federal Register** on February 17, 1979 (43 FR 7174).

The work plan for the Work Group on Risk Assessment, appearing at 43 FR 7195, provides that the general goal of the work group is to characterize the types of health hazards that may result from human exposure to chemicals, devices, consumer goods, and other articles and substances. The initial task established by the work group was to address the problems associated with health risks due to exposure to chemicals, specifically the risk of cancer. The work group set out to examine the available scientific methods used in the assessment of carcinogenic risk and select for use by the four agencies those currently having the strongest experimental and theoretical support. The work group explicitly restricted its task to the development of concepts and methods for assessing risk, without making any attempt to make statements regarding the appropriate regulatory response for particular types and levels of risk. Recently, the Food Safety and Quality Service of the Department of Agriculture joined the other agencies as a participant in the IRLG.

The Report

The full text of the report, entitled "Scientific Bases for Identification of Potential Carcinogens and Estimation of Risks," is set forth in the Appendix to this notice. The report describes (1) the basis for making a qualitative evaluation of whether a particular substance presents a carcinogenic hazard and how the results of epidemiological studies and animal bioassays, along with other types of information, are used in making that evaluation; and (2) the methods that are used in making quantitative estimates of the carcinogenic risk posed by the substance, if such risk estimates are appropriate or required. It represents the best judgment of scientists at CPSC, EPA, FDA, and OSHA and of the participating senior scientists at NCI and NIEHS on the scientific principles applicable to identifying and evaluating substances that may pose a risk of cancer to humans. Scientists at FSQS have reviewed the report and concur. The report is intended to serve as a valuable scientific reference which may be considered by the agencies, consistent with their statutes and in association with other relevant information, in the evaluation of risk and as a means of ascertaining the adequacy of experimental and epidemiological methods used in that evaluation.

The identification and evaluation of carcinogens is a fundamental step in any regulatory program. However, each of the agencies publishing this document for comment administers different laws requiring a variety of findings precedent to regulatory action. It is not the purpose of this notice and comment procedure to give the principles in this document the force of law in making any of those required findings. In the event any of the agencies wishes to utilize this document to develop a substantive rule of law, it will initiate appropriate proceedings under its own applicable statutes. The report does not have any regulatory status at this time other than as a valuable scientific appraisal of scientific principles applicable to identifying and evaluating potential human carcinogens. Accordingly, this notice does not request comments related to the regulatory status of the report.

Scientific and Public Review of the Report

As stated above, the report represents the best judgments of scientists in the IRLG agencies and of the participating senior scientists at NCI and NIEHS. In order better to enable the scientific community at large to review and comment on the report, the agencies are subjecting the documents not only to review through this **Federal Register** publication, but also to scientific peer review through publication in the *Journal of the National Cancer Institute*. The report has been accepted for publication in the *Journal* and is expected to be published in the near future. The process of scientific peer review will proceed concurrently with this notice and comment proceeding.

As previously stated, the report was prepared by personnel in three of the agencies publishing this notice—CPSC,

EPA, and FDA—and by personnel in OSHA, NCI, and NIEHS. The participation of FSQS in the IRLG began after the report was prepared. Because OSHA already has conducted an extensive public proceeding, including a lengthy public hearing, on its proposed rule for the Identification, Classification, and Regulation of Toxic Substances Posing a Potential Occupational Carcinogenic Risk [42 FR 54148, October 4, 1977] and will soon issue a final rule, only CPSC, EPA, FDA, and FSQS are participating in this notice and comment procedure.

Interested persons are invited to submit, on or before September 30, 1979, written comments regarding the report. The comments will be reviewed by the four agencies with the assistance of members of the Work Group on Risk Assessment of the IRLG and scientists at NCI and NIEHS. Comments and any accompanying material should be addressed to IRLG, Room 500, 1111 18th Street, N.W., Washington, D.C. 20207. Comments received after the close of the comment period will be considered to the extent practicable.

Dated: June 26, 1979.

For the Consumer Product Safety Commission:

Susan B. King, Chairman.

For the Environmental Protection Agency:

Douglas M. Costle,

Administrator.

For the Food and Drug Administration:

Donald Kennedy,

Commissioner.

For the Food Safety and Quality Service:

Carol Tucker Foreman,

Assistant Secretary of Agriculture.

Scientific Bases for Identification of Potential Carcinogens and Estimation of Risks

Report of the Interagency Regulatory Liaison Group, Work Group on Risk Assessment

During the preparation of this document, the Interagency Regulatory Liaison Group consisted of four agencies: the United States Consumer Product Safety Commission (CPSC); the United States Environmental Protection Agency (EPA); the Food and Drug Administration (FDA) of the United States Department of Health, Education, and Welfare; and the Occupational Safety and Health Administration (OSHA) of the United States Department of Labor.

Work Group Members [1]

Eula Bingham, *IRLG Principal* (Assistant Secretary of Labor for Occupational Safety and Health)

Joseph V. Rodricks, *Chairman* (Food and Drug Administration)

Elizabeth L. Anderson (Environmental Protection Agency)

David W. Gaylor (Food and Drug Administration, National Center for Toxicological Research)

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Anson M. Keller (Occupational Safety and Health Administration)

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Additional Participants in the Work Group

Roy E. Albert (Environmental Protection Agency)

Richard R. Bates (National Institute of Enviornmental Health Sciences)

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Marvin A. Schneiderman (National Cancer Institute)

ABSTRACT—Three types of evidence can be used to identify substances that may pose a carcinogenic hazard; these types are designated in Part I of this report as 1) epidemiologic evidence derived from studies of exposed human populations, 2) experimental evidence derived from long-term bioassays on animals, and 3) supportive or suggestive evidence derived from studies of chemical structure or from short-term or other tests that are known to correlate with carcinogenic activity. Part II delineates the scientific bases for accepting evidence from these three sources and describes their relative contributions to the determination that a substance may pose a carcinogenic hazard. Further, it details the factors that should be considered in the evaluation of experimental and epidemiologic data for ascertaining the reliability and scientific merit of each source of evidence. It also specifies how certain types of limitations in data may require qualification of conclusions. Because data on experimental animals are currently the major source of information for assessing carcinogenicity, they receive the greatest emphasis. Features of experimental design and conduct that influence the evaluation of such studies are discussed, as are the criteria for making evaluations. The report is not intended to specify how such studies should be designed and conducted; rather, it discusses how data from experimental animal studies of widely varying content and quality should be evaluated for purposes of identifying carcinogens. Epidemiologic data and some of their limitations are discussed in less detail. Chemical structure and the short-term tests that correlate with carcinogenic activity are briefly described, as are their roles in providing suggestive or—if coupled with positive data on animals or humans—supportive evidence of carcinogenicity. In Part II are presented the criteria used to ascertain the adequacy of evidence purporting to show that a substance does not pose a risk of cancer. Part II also includes discussions of some types of experimental evidence that, if the extent and quality are adequate, may be used to show that certain carcinogenic responses observed in experimental animals may not be predictive of human response. Part III sets forth current methodologies for quantification of risk. Included are discussions of mathematical models available for extrapolation, within a biologic system, of cancer incidence data observed at experimental dose levels to estimate risks at the (usually much lower) levels that are of concern for humans. Also presented are the factors that should be considered in attempts to identify the human population(s) at risk and to define their conditions and levels of carcinogen exposure. Part III also deals with correlation of the magnitude of effects observed in one human population group or in experimental animals (under their conditions and level of exposure) with the magnitude of effects in the human population for which the estimate of risk is being made. Limitations in current risk estimation methodologies are described, as are the problems of ensuring that human risk is not underestimated. The issue of thresholds for carcinogens is discussed in the final section of Part III.

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[1] Valuable guidance was received from Arthur C. Upton (Director, National Cancer Institute) and David P. Rall (Director, National Institute of Environmental Health Sciences). The Work Group acknowledges the assistance of Edward Allera (Food and Drug Administration); Ann Barton, Steven Jellinek, Richard Hill, and Ellen Siegler (Environmental Protection Agency); Steven Bayard, Donald Clay, and Raymond Woltman (Consumer Product Safety Commission); Charles C. Brown and James Sontag (National Cancer Institute); Carl Gerber (Office of Science and Technology Policy, Executive Office of the President); Nathan J. Karch (Council on Environmental Quality, Executive Office of the President); and J. William Lloyd (Occupational Safety and Health Administration).

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Part I. Introduction

This document describes the best judgments of the scientists in the agencies comprising the Interagency Regulatory Liaison Group (IRLG) on the scientific concepts and methods currently in use to identify and evaluate substances that may pose a risk of cancer to humans. These are fundamental steps in any program regulating carcinogens. The document was prepared by the Risk Assessment Work Group of the IRLG agencies and senior scientists from the National Cancer Institute (NCI) and the National Institute of Environmental Health Sciences.

The document describes 1) the basis for qualitative evaluation whether a particular substance presents a carcinogenic hazard and how the results of epidemiologic studies and animal bioassays, along with other types of information, are used in making that evaluation; and 2) the methods used for quantitative estimates of the carcinogenic risk posed by the substance, if such risk estimates are appropriate or required.

This document will provide a valuable scientific tool, to be considered with other information, in the evaluation of risk and ascertainment of the adequacy of experimental and epidemiologic methods used in that evaluation. It is an important step in ensuring that the regulatory agencies evaluate carcinogenic risks consistently. The IRLG agencies caution, however, that this document presently has no regulatory status. Its use will, of course, depend upon the statutory requirements of the individual agencies.

The agencies have subjected this document to scientific peer review through the submission of the document to the *Journal of the National Cancer Institute*. In addition, a public notice and comment procedure is initiated by this publication in the **Federal Register.** Since the Occupational Safety and Health Administration (OSHA) has already received extensive public comment on these and other issues regarding the development of its cancer policy rulemaking and will soon promulgate its policy, only the Consumer Product Safety Commission (CPSC), the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the Food Safety and Quality Service (FSQS) will participate in the public notice and comment procedure on this document. At the conclusion of the notice and comment procedure, OSHA will consider whether revisions to its final cancer policy are appropriate. The four agencies emphasize that the goal of this process is to articulate a consistent policy on the scientific principles applicable to the identification and evaluation of substances that may pose a carcinogenic risk to humans.

Part II discusses the qualitative determination that a substance poses a carcinogenic hazard. Part III discusses quantitative estimation of risk.

Part II. The Qualitative Determination That A Substance Poses A Carcinogenic Hazard

The methods used for regulatory purposes in making a qualitative determination that a substance poses a carcinogenic hazard to humans are based on a substantial scientific consensus that has emerged from experience, research, debate, and review. Although some points need further clarification and definition, substantial agreement exists among the Federal regulatory agencies on criteria for evaluating the carcinogenicity of a substance.

In addition to determining that a substance may pose a hazard of cancer, regulatory agencies must consider other possible health hazards, and in some instances they are required to balance considerations of risk with other factors (such as possible health benefits or economic costs and benefits) in reaching regulatory decisions.

DEFINITION AND EXTENT OF THE PROBLEM

Nature of Carcinogensis and Carcinogenic Responses

The characteristic toxicologic event in carcinogenesis is a change in the regulatory mechanism of the target cells, resulting in self replicating cell lesions. The carcinogenic event so modifies the genome and/or other molecular control mechanisms in the target cells that these can give rise to a progeny of permanently altered cells. This progeny of cells constitutes the basis of the neoplastic disease. The expression of the toxic injury therefore does not derive from the same cells originally hit by the toxic agent nor from their functional products but rather from the proliferation of a new population of altered cells.

The critical molecular injury caused by specific carcinogens may be quantitatively extremely limited—even to a few cells—and may therefore not be detectable. What will make it manifest, through the subsequent growth of a clinically detectable neoplasm, is the proliferation of the altered cell population. The intensity of the pathologic response in a subject (i.e., the growth rate and spread of a cancer) depends on conditions of the host subsequent to the initial carcinogenic event and can be modified by other factors, such as enhancing agents and dietary factors. The continued progression of clinical manifestations of the carcinogenic process can occur in the absence of continued exposure to the carcinogen. Carcinogenic effects are therefore self-replicating toxic effects different from the common terminal toxic effects in which the manifestations of toxicity are due to altered functional products, degenerative changes, or death of the target cells themselves *(1)*.

A rigorous methodology must be followed in obtaining, reviewing, and documenting the data required for a determination of carcinogenicity from observations on humans and experimental studies. Both epidemiologic observations and experimental studies need to be correlated with information on the chemical and physical nature of the agents under consideration, their reactivity, and their fate in the environment and in the exposed

organisms. Evidence of carcinogenicity can be obtained from three sources:

1) epidemiologic evidence from exposed human populations;

2) experimental evidence form long-term bioassays in animals;

3) suggestive evidence derived from studies of chemical structure, reactivity, DNA damage and repair, mutagenicity, neoplastic transformation of cells in culture, induction of preneoplastic changes, or from other short-term tests that correlate with carcinogenicity.

In the evaluation of the results of carcinogenesis studies, the evidence obtained from epidemiologic observations or from experimental bioassays does not necessarily fall sharply into the two categories of positive and negative: In many instances the evidence may be insufficient for a definitive assessment.

Estimation of the Number of Carcinogenic Substances

Relatively few chemicals have been found to be carcinogenic. In fact, available evidence indicates that most substances do not cause cancer. The NCI's "Survey of Compounds Which Have Been Tested for Carcinogenic Activity" *(2–8)* and other literature surveys and reviews provide results of long-term animal bioassays on about 7,000 chemicals. Evidence of carcinogenicity on the basis of currently accepted experimental testing methods is available for less than 1,000 chemicals and possibly for as few as 600–800, *(9–34)*. Many of these substances were selected for testing because of their structural similarity to known carcinogens. Thus these data considerably overstate the true proportion of carcinogenic substances in the human environment. A critical review of the literature on carcinogenicity of chemicals has been undertaken by the International Agency for Research on Cancer (IARC) with the support and collaboration of NCI *(9–25)*. Of 368 chemicals evaluated in volumes 1–16 of the IARC monographs, some evidence of carcinogenicity was found for 247 *(35)*.

A small number of chemicals has been adequately studied by epidemiologic methods to determine whether a carcinogenic hazard exists. By one recent estimate, 26 chemical substances or processes have been identified as responsible for cancer induction in humans *(9–25, 35)*. Of those 26 substances, 6 were first identified as carcinogenic by tests in animals, whereas 20 were first identified by epidemiologic evidence.

Of the 368 substances for which carcinogenesis data were reviewed by the IARC, 221 showed some evidence of carcinogenicity from tests in animals, but these substances had not received adequate epidemiologic study to evaluate their effects in humans *(35)*. In addition, 15 occupational categories have been reported to be associated with excess cancer incidences without identification of a specific etiologic agent *(36–50)*.

Enhancing Factors

Experimental and epidemiologic data suggest that some agents may not be carcinogenic alone but substantially contribute to the development of cancer in subjects that have been exposed to carcinogens. Depending on experimental circumstances, these agents have been referred to as cocarcinogens, promoting agents, syncarcinogens, or more generally, modifying or enhancing factors *(51, 52)*.

Research on this category of agents suggests that they may work through a number of mechanisms of action, including *(51, 52): a)* alteration of the uptake and/or distribution of carcinogens, *b)* modification of the metabolic activation of carcinogens, *c)* enhancement of the susceptibility of target tissues, and *d)* acceleration of neoplastic progression.

Current evidence suggests that some of these agents act by a mechanism that may be specific for particular organs or conditions of exposure. Because of the possible specificity of their mechanisms of actions, the activity of these agents may not be recognized by conventional bioassays. Since no common general pathway of action has been recognized, it is not expected that tests based on a single-mechanism end point will be applicable for the identification of a broad range of these substances.

Enhancing mechanisms may be a major factor in the development of human cancers; therefore, their identification and control may be important in cancer prevention. Since no general methodology yet exists for testing and evaluation of this entire group of substances, the special circumstances under which each may act must be carefully evaluated. Interpretation of a positive effect in a carcinogenesis bioassay as being due to one of these mechanisms would require rigorous documentation that a full carcinogenic process is not involved.

Variability of Effects of Carcinogens

Variability in the action of carcinogens may be due to inherent differences in susceptibility among species and strains of test animals and within populations of humans, and also to variability in the intrinsic differences in carcinogenic reactivity of individual agents. For example, aflatoxin B_1 is strongly carcinogenic in rats but is ineffective in several strains of adult mice *(53)*. β-Naphthylamine is carcinogenic for humans, dogs, and several other species, but this compound has not produced tumors in rats *(54)*. With some other carcinogens, there is a greater concordance of results among species: Dimethylnitrosamine has been found to be carcinogenic in all of the strains of vertebrates tested *(55)*.

Species and strain differences in susceptibility to carcinogens may be due to factors that affect transport and metabolism, which in turn determine the effective dose of the ultimate form of the carcinogen delivered to target cells. These differences may also be due to inherent variations in susceptibility to neoplastic transformation of different organs in different species (*56*).

Differences in the level of carcinogenic effect of individual agents can only be compared with precision under strictly defined conditions of dosage and biologic end points. Frequently the level of effects, even under strictly defined conditions, will show marked variability depending on the test system used. Nevertheless, in the extreme, some carcinogens are clearly more effective than others by several orders of magnitude (*9–25*). However, such comparative potency estimates must be made with caution.

EPIDEMIOLOGIC EVIDENCE

Evidence of carcinogenic activity of an agent can be obtained from epidemiologic studies when evaluation of the observations shows that the test agent causes an increased incidence of neoplasms or a decrease in their latency period.

Evidence from studies of human populations identifies carcinogenic chemicals to which those populations were exposed in the past. Many substances that have been identified as carcinogens in humans were discovered by epidemiologic studies of exposed workers; this evidence dates from 18th-century observations of cancer in chimney sweeps to more recent observations on dye workers, asbestos workers, and workers in certain chemical industries (*31*). It was noted early that clinical signs of cancer are delayed for a long time after initial exposure to carcinogens. This period of latency—often 5–40 years from initial exposure until the disease appears—makes prompt detection of newly introduced carcinogenic substances by epidemiologic studies nearly impossible.

As more substances are introduced into the human environment and as

more are tested experimentally, it is expected that a larger proportion will be identified as carcinogenic; this will be followed by adequate control measures, so that epidemiologic confirmation may become impossible.

Types of Epidemiologic Evidence

Types of epidemiologic evidence of carcinogenicity in humans include neoplastic response directly related to duration and dose of exposure, incidence or mortality differences related to occupational exposure, incidence or mortality differences between geographic regions related to environmental rather than genetic differences, altered incidence in migrant populations, time trends in incidence or mortality related to either the introduction or removal of a specific agent from the environment, case-control studies, and the result of retrospective-prospective and prospective studies of the consequences of human exposure. Clinical case reports may also provide early warning of a potential carcinogen (*57*).

The two main types of epidemiologic studies used to establish evidence of a carcinogenic hazard are cohort studies and case-control studies (*58*). Epidemiologic cohort studies involve the comparison of groups differently exposed to a substance. The comparison may include *a*) totally unexposed versus exposed groups, *b*) groups having distinctly different levels of exposure, or *c*) rates in exposed groups versus rates prevailing in the general population. The groups need to be comparable for demographic factors such as age, sex, and race, and controlled for exposure to known carcinogens.

Epidemiologic case-control studies involve comparison of people with a given cancer type versus people without the disease but otherwise comparable with respect to appropriate demographic variables, to ascertain if they differ in exposure to the cancer hazard under investigation.

Epidemiologic findings gain greater force with increasing numbers of well-conducted studied that show similar effects from a given substance under different circumstances.

Absence of a positive statistical correlation does not by itself demonstrate absence of a hazard. Whereas negative epidemiologic data usually do not adequately establish the noncarcinogenicity of suspected materials, such negative data obtained for a given agent from epidemiologic studies of sufficient extent and duration may indicate the upper limits for the rate at which a specific type of exposure could affect the incidence and/or mortality of specific human cancers under the conditions of observation.

The detectability of a carcinogenic effect in a group of humans depends on several factors, including the duration and extent of exposure, size of the exposed population, and background rate of cancer in the target organ. Evaluation of epidemiologic studies requires a knowledge of the smallest possible increase in tumor incidence detectable under the conditions of each study. Such information has rarely been included in published reports. This information is, however, of critical importance in the evaluation of apparently negative studies.

The larger the number of persons in the exposed and control groups and the greater the similarity of these groups for factors other than exposure to the suspect carcinogen, the more likely will an effect be detected. Often, only a small number of humans exposed to a substance can be studied, conditions of exposure are inadequately defined, and records are incomplete. Thus a carcinogenic effect can be easily missed by epidemiologic methods, especially when common types of cancer (such as cancer of the lung, breast, colon, or rectum) are studied, inasmuch as these types often require a large excess of risk before a causal relationship can be identified for the exposure to a particular substance. Substances distributed widely in commerce or in the environment are particularly difficult to study by epidemiologic methods unless high risk ratios are observed, because it is often impossible to identify unexposed groups as controls or to separate groups with high and low exposure. The problem of adequate controls is further compounded by the long latency of cancer, during which multiple opportunities exist for exposure to other potentially carcinogenic substances and modifying factors. The effects of such other exposures on rates of cancer are rarely known, although in some instances they were found to be more than additive (*22*).

Disease Ascertainment

Because the effect under consideration is cancer morbidity or mortality, it is important to establish the validity, consistency, and reliability of the methods used to ascertain that neoplastic disease is clinically present or that it causes death.

Disease classification is also important, and uniform criteria of tumor nomenclature are needed. Some types of cancer may be classified under a generic name in such a way that changes in their frequency may be missed if only the generic classification is used. Some members of a population may be "lost" to a study if their disease conditions cannot be adequately ascertained.

Specific uniform procedures are not recommended here, but careful attention needs to be given to the extent to which these problems may affect comparison of relevant characteristics between groups.

In the statistical evaluation of cancer incidence or mortality differences, there has been a strong tendency for particular confidence levels (e.g., 95%) and particular probability values (e.g., $P=0.05$ or $P=0.01$) to be used as standard points for a finding of statistical significance. It is recognized that probability values fall along a continuum and should be so reported. The uniform use of a standard probability value is not suggested. Regulatory needs are best served by accurate estimates of the possible role of chance in accounting for observed differences.

The most important parameter in the assessment of an epidemiologic study is the magnitude of the effect measured; its interpretation is tempered by considerations of biologic plausibility, bias, confounding factors, and chance.

EVIDENCE FROM EXPERIMENTAL ANIMALS

Evidence of the carcinogenic activity of an agent can be obtained from bioassays in experimental animals showing that the test substance causes either an increase in the incidence of neoplasms or a decrease in the latency period.

The experimental design and conduct should be reviewed for quality and accuracy, and the results should be evaluated statistically for significance, with the only major experimental variable between control and experimental groups being the presence of the test substance. Positive results observed in more than one group of animals or in different laboratories and the demonstration that the occurrence of neoplasms follows a dose-dependent relationship provide additional confirmation of carcinogenicity. Determination that a causal relationship exists between a test treatment and the responses observed in a bioassay is a complex judgmental activity that includes evaluation of the identity of the test agent and the biologic test system, the conditions of exposure, the methods of observation, and the qualitative and quantitative nature of the pathologic response. The assessment of carcinogenicity therefore relies upon the

judgment and experience of professionals. The following discussion refers to aspects of experimental design and conduct that concern evaluation of results. They are not intended as a prescription of protocols.

Criteria for Evaluation of Experimental Design and Conduct

Experimental Design

Commonly recommended requirements for a thorough assessment of carcinogenic potential in experimental animals generally include *a*) two species of rodents, *b*) both sexes of each, *c*) adequate controls, *d*) a number of animals sufficient to provide an adequate resolving power to detect a carcinogenic effect, *e*) treatment and observation extending to most of the lifetime of the animals at a dose range including one level likely to yield maximum expression of carcinogenic potential, *f*) detailed pathologic examination, and *g*) statistical evaluation of results (*9–25, 27, 31, 32, 57, 59–73*).

Positive results obtained in one species only are considered evidence of carcinogenicity. Positive results in more limited tests (e.g., when the observation period is considerably less than the animal's lifetime), but by experimentally adequate procedures, are acceptable as evidence of carcinogenicity. Negative results, on the other hand, are not considered evidence of lack of a carcinogenic effect, for operational purposes, unless minimum requirements have been met.

Choice of the Animal Model

The animals used most often for carcinogenesis bioassays are mice, rats, and hamsters. These animals are used extensively because 1) their natural life-spans are short; 2) they are easier to breed and handle in large numbers than larger animals; 3) they are inexpensive and easy to care for; 4) inbred strains exist that are genetically homogeneous for such traits as "background" cancer rates, susceptibility to carcinogens at specific organ sites, longevity, and response to husbandry systems. Adequately designed and performed studies in other mammalian species may also provide useful information on carcinogenicity. For human risk evaluation, data obtained from bioassays with the use of nonmammalian species can presently provide only suggestive evidence if positive but permit no conclusion if negative.

Experience on the background incidence of tumors in the colony of animals used for testing, obtained over a period of years by extensive observation of untreated animals under the same general maintenance conditions (historical colony controls), is useful in assessing the relevance of experimental findings, such as the appearance of rare tumors.

Rodents with different types of genetic homogeneity have been used for carcinogenesis bioassays. These include *a*) inbred strains, *b*) first-generation hybrids of parents of inbred strains, *c*) randombred animals from a closed colony, *d*) noninbred animals, and *e*) animals of unspecified strains or origins. As the genetic and/or environmental variation increases, so does the need for concern about the variation of background tumor incidence.

A particular problem is posed by the use of certain strains of rodents in which particular tumor types reach a high frequency, often well above 50%, in untreated controls. Examples of such strains include mice of strain A for lung adenomas, strain AKR for lymphomas, strain C3H/HeN males for liver cell tumors and C3H females for mammary tumors, and females of several rat strains for mammary fibroadenomas. Although viral factors have been identified in the etiology of mouse AKR leukemia and C3H mammary tumors, no such factors are known to be at work for the other types mentioned above. The effect of carcinogens has been clearly demonstrated in all of the above strains by detection of substantial decreases in the latency period, by definite increases in incidence or multiplicity of these tumor types, and by the induction of tumors of other histologic types in the same or other organs (*2–25*). Caution must be used, however, in evaluating the significance of a higher incidence of these tumors in a treated group compared with concurrent controls when the incidence in the treated animals falls within a range commonly seen in historical controls from the same colony.

Background incidence rates for tumors of the lung, liver, mammary gland, and hematopoietic tissues are much lower in many other strains of mice, and for tumors of the mammary gland in other strains of rats. In these other strains, no unique biologic trait distinguishes the types of tumors mentioned above from many others, and no reason has been demonstrated for considering that they have any different significance than tumors in other organs as indicators of a carcinogenic response, under otherwise appropriate test conditions.

Number of Animals

The number of animals in each group to be effectively considered for the evaluation of carcinogenesis test results is the number in which detection of carcinogenic effects could be expected. This number is obtained by subtracting from the number of animals started on the test the number of those lost to adequate observation (e.g., by intercurrent death followed by cannibalism or autolysis). The number of animals on which complete pathologic examination is conducted is important in the evaluation of tumor pathology.

Positive results can be obtained in tests with the use of a small number of animals if the test is otherwise adequately designed and conducted and if the tumor response is significant. For example, in a group of 15 animals, if 12 show a well-defined neoplastic lesion of a kind rarely seen either in matched or historical controls, the finding is positive. However, a negative finding in a group of 15 animals is not adequate evidence that the test agent is not carcinogenic.

Ideally, the number of animals required to provide adequate negative evidence would be such that an excessive risk would not arise if the test failed to detect carcinogenicity. The likelihood that such a risk would not arise increases both with the number of animals on test and the extent to which human exposure levels are exceeded. The probability of a false negative finding also depends on the background tumor rate in the control animals. For example, if a one-sided level of statistical significance of 5% is used with 55 animals, there is an 80% chance of detecting a tumor rate of 20% in the treated animals for whom the control rate is 5%, whereas 130 animals are required to detect the same difference if the control rate is 30%. The number of animals tested may need to be increased if the number of humans exposed is large or if a small margin of safety exists between the animal dose and the human exposure.

In practice, resource limitations often require a trade-off between the number of animals used and the number of substances tested in order to control the total cancer burden resulting from chemical carcinogens. This is particularly true with substances whose toxicity limits the test dose to a low multiplicity of human exposure levels. In those instances, it may be necessary to accept a lower than ideal degree of "negative evidence."

Route of Administration

A key factor in the comparison of an experimental result to the human situation is to assess whether cells

capable of malignant transformation are exposed to the reactive carcinogenic agent(s) in both the human and the experimental animal, regardless of whether transformation occurs in identical organs and cell types. Although this comparison is most readily made from experiments with animals in which the route of administration is the same as that in humans, other routes of administration may also be comparable and provide results useful for evaluation of the human hazard. For example, some chemicals are rapidly absorbed by inhalation, circulated through the body, and metabolized by the same pathways that occur following intravenous exposure (*74*).

Some routes of administration in animals may fail to provide adequate metabolic activation or exposure of target tissues and therefore may lead to false-negative results. This possibility should be assessed in evaluating negative results obtained when the route of administration in animals differs from the route of human exposure.

Generally, the route should be one that leads to absorption and distribution of the test substance. The induction of tumors at a remote site in the animal is evidence of absorption, distribution, and possible metabolic activation of the test substance. If exposures of both humans and animals involve absorption of the substance, any route of administration in animals may be regarded as relevant for a qualitative demonstration of human hazard unless there is evidence that the route of administration in the test species results in the production of carcinogenic substances (from degradation or metabolism) which does not ever occur with human exposure.

When tumors appear only at the site of injection or implantation, careful review is necessary. If there is reason to believe that the tumors occur as a result of "solid state" carcinogenesis (*75, 76*), the results may be inappropriate for extrapolation to human exposure. If, however, the test material produces tumors at the site of injection or implantation as a result of its chemical reactivity, this response is an indication of carcinogenicity.

There are a number of practical reasons for studying certain substances in animals by a route of administration different from the expected route of human exposure. If a substance under test is highly volatile, accurate administration in food may be difficult because of evaporation; often feeding through a stomach tube is used so that the dose may be measured with greater accuracy. Even for nonvolatile test substances, a stomach tube may be used when it is important to know the exact amount of a substance administered to the test animals. The administration of high doses of a test substance with a disagreeable odor or taste may require the use of routes other than ingestion.

Thus experimental exposures need not necessarily be by the route of human exposure in order to be meaningful, but possible physiologic and metabolic differences related to routes of absorption and distribution should be considered in the assessment of their relevance.

Identity of the Substance Tested

Substances to which humans are exposed through their occupations, the environment, and the products they use vary widely both in the number and the proportion of contaminating impurities. A full assessment of the carcinogenicity of an impure mixture ideally requires that each component be tested individually at an adequate dosage and that the mixture itself be tested in order to detect cumulative or synergistic effects. Limitation of resources makes this ideal approach impractical as a routine. It is common, therefore, simply to rely on tests either of the product to which humans are exposed, including the impurities present, or of the purified principal chemical substance(s). Because the products may vary according to procedures used in manufacture and processing, tests for one commercial product may not be applicable to another product containing a different set or level of impurities. Change in the manufacturing process of a product may require additional tests to confirm the safety of the new product if the change involves the introduction of different impurities or a substantial increase in the amount of any single component of the product. Even though it is accepted practice to test mixtures, the nature of any impurities known or likely to be present as a result of the manufacturing process is important and may require separate examination or testing. Information on the carcinogenicity of any single chemical in a mixture is an indication of potential hazard of the entire mixture. However, negative results obtained on a component of a mixture may not reflect the potential carcinogenicity of the entire mixture.

Dose Levels

"Testing should be done at doses and under experimental conditions likely to yield maximum tumor incidence." This recommendation of an FDA advisory committee summarizes the issue of test doses (*68*).

Bioassays with the use of a few dozen or even a few hundred animals have relatively low sensitivity for detection of carcinogenic effects. Millions of people of varying degrees of sensitivity or exposure may be exposed to the substances under evaluation. Although a test animal cannot be strictly viewed as a "surrogate" of a large number of people without oversimplification, the role of animal tests is to provide maximum detectability of carcinogenic effects within the already narrow confines of test sensitivity. Under otherwise identical conditions, the greater the ratio of test exposure to human exposure, the greater is the safety margin provided by a negative result in a carcinogenesis bioassay.

It is generally recommended that more than one dose level be tested. Most carcinogenic effects show a positive dose-response relationship, but maximum tumor incidence in test animals may not occur at the highest dose when competing toxicity prevails. The highest test dose that can be effectively used in a carcinogenesis bioassay is limited by the conditions of absorption, by the amount that the animal can tolerate during lifetime administration without unwanted toxic side effects, and by the effects on nutrition when the chemical constitutes too large a proportion of the diet.

Results of bioassays done at doses and under conditions permitting maximum expression of carcinogenicity provide a sound basis for the identification of a carcinogenic hazard or its absence.

It is important to estimate the highest dose level that will be tolerated by the test animals during lifetime admistration, i.e., the estimated maximum tolerated dose (EMTD). The EMTD is defined as the highest dose that can be administered to the test animals for their lifetime and that is estimated not to produce *a*) clinical signs of toxicity or pathologic lesions other than those related to a neoplastic response, but which may interfere with the neoplastic response; *b*) alteration of the normal longevity of the animals from toxic effects other than carcinogenesis; and *c*) more than a relatively small percent inhibition of normal weight gain (not to exceed 10%) (*71*).

The EMTD is determined on the basis of prechronic tests and other relevant information. If the test reveals that the EMTD is too high to meet the conditions defined herein, positive results obtained above the EMTD are acceptable as evidence of carcinogenicity unless there is convincing evidence to the contrary. Alternatively, negative results obtained

above the EMTD are considered inadequate unless particularly strong and specific scientific reasons justify their acceptance as negative. Positive results obtained at or below the EMTD provide evidence of carcinogenicity.

Age at Treatment

Because of the long latency period required for induction and manifestation of tumors, treatment should be started in young animals, and the animals should be observed for a carcinogenic response through most of their expected life-spans. The older the age at first treatment, the shorter is the remaining life-span available for tumor development; consequently, the smaller is the chance of detecting delayed carcinogenic effects.

Although treatment is often started in young adult animals soon after weaning, some protocols call for treatment soon after birth (neonatal) or during fetal development (transplacental). The rationale for exposing test animals transplacentally or neonatally is based on the greater susceptibility of certain organs to carcinogens during early development. Such susceptibility has been demonstrated in several species, including those commonly used for bioassays (*77, 78*). Animals first treated during the perinatal period must be also treated and observed throughout their life-spans to obtain a valid negative response.

Virtually any agent that is carcinogenic in adult animals can be expected to have some carcinogenic effect when administered to young animals including the neonate and the fetus. Unless a substance is demonstrated to be exclusively carcinogenic when administered to the fetus or neonate, enhanced perinatal susceptibility to carcinogens should be considered not a separate and distinct toxicologic property; rather, it should be a means for increasing the sensitivity of conventional bioassay procedures by extension of the exposure period to these earlier and more susceptible portions of the life-span.

It should be emphasized that these protocol modifications greatly complicate dose selection and experimental design. An agent may be significantly more toxic to the fetus, the neonate, or the pregnant or lactating female animal than to the normal young adult of either sex. This requires independent determination of the toxicity and EMTD. Furthermore, individuals in the litter of a treated pregnant animal cannot be considered independent units for statistical evaluation of effects.

Conduct and Duration of Bioassays in Animals

A long-term bioassay for carcinogenesis in animals is a complex procedure requiring control of many variables for several years. Professional experience and knowledge of the relevant biologic parameters are needed for adequate quality control. Detailed guidance on procedures is provided by reports such as the FDA's "Good Laboratory Practice Regulations" (*79*) and the NCI's "Guidelines for Carcinogen Bioassays in Small Rodents" (*71*).

Review of the observations made during the bioassay (on food intake, weight, clinical course, and pathologic conditions of the animals) provides a basis for determining whether these experimental variables are recorded in sufficient detail and are internally consistent to permit independent assessment of their validity.

The purpose of these bioassays is primarily to provide maximal opportunity for detection of a neoplastic response; therefore, the longer the period of observation the better is the chance of detecting delayed effects. A "point of diminishing return" can be reached when intercurrent disease and/or survival considerations make the observation or evaluation of old animals particularly difficult. It is expected that the animals will be observed for most of their life-spans. The best negative evidence for the carcinogenicity of a substance is obtained from tests in which both exposure and observation last through all or nearly all of the expected life-spans of the animals under study.

Negative results decrease in value as the exposure and observation periods are shortened, and they become practically meaningless if these periods are shorter than half the life-spans of the animals. When some animals die early in the course of a test, the value of the test is reduced as a function of the percentage of animals dying without tumors at periods markedly shorter than the life-span of the species. Sometimes, a positive carcinogenic response may be definitely demonstrated in a shorter period of observation if the experiment is adequately controlled; in such cases the test is considered valid even if it is shorter than usual (*80*).

Accepted procedures include *a*) the observation of all animals in the study (treated and control groups) until their spontaneous death, *b*) the sacrifice of animals that show clinical signs of severe illness or impending death (sacrifice of moribund animals prevents losses due to autolysis and provides better observation of tissue pathology), and *c*) terminal sacrifice at a scheduled date near the end of the life-span (e.g., after 24 months on test).

Criteria for Evaluation of Pathology

Pathology Examination

The evaluation of carcinogenesis bioassay results rests on the extent and accuracy with which organs and tissues of both treated and control animals are examined for morphologic changes. After the termination of a bioassay, the only physical evidence that can be used to permit reevaluation of results, even years afterwards, is represented by the written descriptive and diagnostic records, the graphic or photographic records of gross or microscopic observations, and most importantly, the original slides of tissue sections for microscopic examination. The histologic slides are of critical importance as a lasting direct documentation of the conditions of normal and abnormal tissues and organs, both for scientific and regulatory purposes. Quality and extent of pathologic documentation are therefore major factors in establishing the validity of bioassays in animals (*71, 79*).

Although a well-conducted pathologic examination cannot generally rescue a poorly designed or badly conducted bioassay, inadequate pathologic examination can significantly reduce or eliminate the value of an otherwise well-conducted experiment. Among the factors to be considered in evaluation of the pathologic examination are:

1) the care and thoroughness of gross tissue examination and the qualifications of the persons conducting this examination to recognize abnormalities;

2) the quality of preservation, sectioning, and staining of tissues;

3) the accuracy of the record-keeping system used for labeling tissues as they are moved from the animal through slide-processing to final diagnosis and reporting;

4) the extent of selection of normal and abnormal tissues for microscopic examination; and

5) the qualifications of the pathologist making the microscopic examination.

The numbers of tumors or other lesions diagnosed by the pathologist are not a thorough assessment of incidence unless each factor is adequately considered, controlled, and documented.

The strength of evidence provided by a bioassay also depends on the number of tissues examined. Failure to observe excess tumors in treated animals cannot be considered evidence of the absence of a carcinogenic hazard unless all

organs have been examined grossly and all grossly visible suspect lesions have been examined microscopically. In a large organ, the taking of a single random section for histologic examination can result in failure to detect small tumors. Thus multiple cuts through such organs should be made.

It is also important to open and search the entire cavity of all hollow organs for abnormalities. For example, the entire length of the gastrointestinal tract should be opened and inspected. Grossly visible lesions should be selected for histologic examination, and if they are not subsequently observed on tissue slides, preparation of additional sections may be necessary until the gross lesion is verified histologically.

Furthermore, histopathologic examination should be made of major organs in the treated groups and matched controls, and specific organs should be studied in detail in all dose groups and controls in which there is either gross or microscopic evidence of lesions. Major organs are defined in the NCI's "Guidelines for Carcinogen Bioassays in Small Rodents" (*71*). Positive evidence of carcinogenicity may be valid for a particular organ if it has been adequately examined in both treated and control groups. Negative reports are inadequate for any organ that has not received careful gross examination in all animals and histologic examination of suspect lesions. The more limited the number of organs examined grossly and microscopically, the less the value of the experiment in providing evidence of a negative result.

Evaluation of Pathologic Results

The evaluation of bioassay results and their quality requires a detailed review and expert judgment of all the experimental conditions and observations, including the identity of the test substance; the conditions of administration; the identity, source, and characteristics of the test animals; the accuracy and systematic recording of observations; the extent of pathologic examination; and the competence of the investigator. Meticulous and detailed documentation is of great importance.

Several criteria are applied in the evaluation of bioassay results.

1) *Internal consistency of the data* is important in reviewing the conduct of the test. Apparent inconsistencies should be investigated by analysis of records.

2) *Reproducibility of test results* can be demonstrated within a single experiment (in different groups of similarly treated animals or in different dose-level groups) or in separate bioassays conducted with the same experimental design in the same or in different laboratories. Evidence of reproducibility adds greater confidence to the evaluation of results. Statistical considerations provide an estimate of the level of detectability of an effect and the consequent level of probability that the effect may be missed in a repetition of the test in a given number of animals. Apparent contrary results in any two tests may be simply an effect of chance variation and may be fully compatible with an identical mechanism and level of activity of the test compound.

3) *Evidence of a positive dose-response relationship* adds further confidence to the evaluation of a positive test, but lack of it may be due to testing in a portion of the dose-reponse curve with a shallow slope or even with a declining slope due to competing risks. In the presence of positive results in well-designed, well-conducted tests, evidence of reproducibility and positive dose-response relationships is not necessary to reach a conclusion of carcinogenicity.

4) *Concordance of results* obtained under differing test conditions (e.g., different species, different routes of administration, or markedly different basal diets) provides greater confidence in the evaluation of both positive and negative studies, but it has a different meaning from "reproducibility" within the same tests or under the same conditions. Lack of concordance from tests performed under different conditions does not, in itself, detract from the validity of the positive test. Reasons for a discordance in observation may be identified by evidence obtained during a test or may be sought through further research.

The response to carcinogens in different animal species and even strains is known tc vary greatly because of genetic, metabolic, nutritional, and other factors that effect susceptibility in a given test animal. Present knowledge indicates that a substance that is clearly carcinogenic in one test species is likely to be carcinogenic in other species, that it may take extensive tests in several species to demonstrate this correlation, and that the responsive target tissues or organs and the types of tumors induced in different species may vary greatly. Therefore, although concordance of positive results (even if different tumor types are involved) adds support to an evaluation of carcinogenicity, the finding of negative results in some other species generally does not detract from the validity of a positive result as evidence of carcinogenicity for the test substance.

In this respect, positive results supersede negative ones. The assessment of such apparent discrepancies in results requires consideration of all experimental variables, since apparently negative results may derive from limitations in the sensitivity of the test (e.g., early scheduled sacrifice, limited extent of pathologic examination, and statistical probability). If the positive result is itself not fully conclusive or if reasons exist for questioning its validity as evidence of carcinogenicity, the result is generally classified as "inconclusive" or "only suggestive" even in the absence of other negative test results.

5) *Evaluation of tumor incidence* is made on the basis of the pathologic findings and therefore depends on professional diagnostic judgment. Tumor incidence is evaluated by consideration of all tumors of specific organ sites or anatomically or physiologically related systems. At present there is considerable uncertainty about the interpretation of carcinogenic responses in terms of the total tumor yield in contrast to the response in terms of a statistically significant increase of tumors in specific target organs or tissues. Traditionally, carcinogens have been recognized in studies on humans and animals by a decisive increase in tumors of target organs. However, it is conceivable that a general increase in total tumor yield, in the absence of an excess incidence in one or more target tissues, could occur—for example, by a promoting effect that generally increases the spontaneous incidence of tumors in test animals or by the action of a multipotent carcinogen whose response did not reach statistical significance in any one organ even at the maximum tolerated dose. In some instances, however, control animals may have a high frequency of tumors at certain sites (e.g., testicular tumors in F344 male rats). In such instances, a simple cumulative count of tumor-bearing versus tumor-free animals may fail to reveal carcinogenic effects in the treated groups. Prudent judgment is needed on the appropriate categorization of tumors used to evaluate induced effects.

A positive result in a carcinogenesis bioassay can be based on evidence of the induction of an increased incidence or a substantially decreased latency period. The latter is often difficult to establish. Determination of the latency period can be made by various techniques of observation during a bioassay. If both test and control animals are sacrificed at a fixed time, only the early part of a temporal distribution curve may be observable; consequently, the estimate of the

average latency period for all tumors or tumor-bearing animals may be artificially altered. If the test and control groups are allowed to live out their life-spans, the comparison of latency periods must take into account the relative survival and the number of animals at risk, particularly in the case of competing risks.

The methods used in estimating the latency period must be defined in the context of each bioassay. It is always difficult to determine the exact onset of a neoplasm. Morphometric criteria may be used for tumors (e.g., skin or subcutaneous tumors) detectable during clinical observation of the animals and a minimum size may be established as a criterion for identification. For neoplasms of the internal organs it is practically impossible to determine an adequate time of onset: Methods such as palpation of the abdomen are highly subjective and generally unreliable. Serial sacrifice studies provide excellent data on time to tumor induction, but they should not be substituted for adequate numbers of animals under lifetime observation. In most instances, what is referred to as latency period is the time between the beginning of the exposure and the observation of a tumor at death. This parameter is obviously influenced by all the factors that determine time of death, e.g., intercurrent diseases, other tumors, or growth rate of individual tumors. Here too, the judgment of experienced pathologists may provide critical evaluation of such aspects as tumor size, location, cell differentiation, and invasion; these factors may contribute to an estimate of temporal sequence.

The observation in treated groups of tumors that are considered rare in untreated and historical controls may raise considerable suspicion even when their incidence is below the required level of statistical significance. Careful review and cautious judgment are necessary in their evaluation; often the rarity of a tumor type is estimated on the basis of a small control population. The occurrence of one or a few neoplasms of a kind, however rare, is not necessarily evidence that a substance is carcinogenic in the absence of other supporting evidence.

6) *Evaluation of tumor morphology* in the final analysis of bioassay results is highly dependent on the way in which pathology data are categorized. It is incorrect, for example, to subdivide diagnoses into so many individual categories based on different stages of disease or different morphologic features that no single category is large enough to be statistically significant. At the other extreme, it is incorrect to group unrelated end points in a way that maximizes the opportunity to find statistical significance, whether or not such groupings are biologically meaningful.

Carcinogenic and chronic toxic effects of a chemical on an organ, tissue, or cell develop through a series of stages from minimal changes to advanced and possibly fatal end points (*81*). The stage reached at any particular time is related to the dose of the substance, the conditions of exposure, the time elapsed since beginning of exposure, and host susceptibility factors. Early lesions that are pathognomonic of a disease process resulting from toxic chemicals should be grouped with more advanced lesions, whether or not the animal has survived long enough for the process to develop to the latest stages. The carcinogenic process may go through early stages including atypical hyperplasia, carcinoma in situ, and/or historically benign tumor before progressing to a clearly malignant stage. Although the stage of development is of critical importance in clinical oncology for assessing the prognosis of a patient at the time of therapy, it is not relevant in deciding whether a chemical is capable of inducing cancer as long as the induction of lesions recognized as neoplastic is conclusively demonstrated.

The induction of preneoplastic lesions in the process of cancer development is an indication that the test substance is capable of inducing cancer in a susceptible host given sufficient exposure and time for cancer to arise. Care must be taken, however, to distinguish atypical hyperplasias that are pathognomonic of neoplastic progression from other nonspecific or reactive hyperplasias.

In the evaluation of bioassays, the concern is with the capability of a test substance to react with a biologic system to give rise to a neoplastic response that may develop through all stages to malignancy. One issue is whether or not the response is the kind that stops at the benign stage and never evolves further to the invasive and metastasizing stage. Few if any tumor types are presently known to belong to this category, which could be called "permanently benign" tumors. For benign tumors, no specific mechanism of induction is known that can be distinguished from the mechanisms of induction of other neoplasms. Moreover, no established body of evidence exists showing that certain substances or groups of substances are capable of inducing exclusively permanently benign tumors without ever inducing any more malignant ones. The mammary fibroadenoma is generally considered to be a benign tumor in both the human (*82*) and the rat (*83*), and it has been suggested that its experimental induction provides little evidence that the inducing agent can cause cancer. X-rays or carcinogenic polycyclic aromatic hydrocarbons, however, which principally induce fibroadenomas in some rat strains, induce mostly malignant adenocarcinomas in other strains; the genetic characteristics of the animal rather than the inducing agent determine whether benign or malignant tumors develop (*84*). Thus the induction of benign tumors, even of a type that rarely progresses to a malignant stage, must be considered a warning that the inducing chemical may be capable of causing cancer in some humans. The induction of benign neoplasms, even if they were demonstrated to be of a permanently benign type, would therefore be considered evidence of carcinogenic activity unless definitive evidence is provided that the test chemical is incapable of inducing malignant neoplasms.

Neoplasms at a benign stage may jeopardize the health and life of the host. Furthermore, it is extremely difficult to rule out the presence of malignant changes simply on the basis of a limited histologic examination of the primary tumor, because focal malignant change or local invasion may have occurred in other areas of the tumor that were not examined microscopically. Similarly, it is very difficult to rule out the metastatic spread of a neoplasm that may be biologically capable of metastasizing without an extremely detailed search for metastases, which can begin as small foci of one or a few cells lodged in the arteriolar walls of peripheral organs (*85*). The frequency of observation of such metastases depends directly on the amount of peripheral tissue that is examined (*86*).

Another case to be considered is the combination of neoplasms diagnosed as benign and malignant. This may include instances in which the incidence of histologically malignant tumors is only a relatively small fraction of the total tumor incidence but represents the most advanced stages of the neoplastic response. Although the number of tumors diagnosed as malignant may not reach statistical significance as such in the number of animals at risk, the total neoplastic response (benign and malignant) may be clearly significant.

Some common types of neoplasms found in carcinogenesis bioassays in laboratory rodents are among those

often diagnosed as being at a benign stage when observed in test animals. Examples include lung adenomas, skin and bladder papillomas, liver cell adenomas (hepatomas), and hemangiomas in various organs. All of these tumor types are known to progress to frank malignant stages. No pathogenetic mechanisms have been identified that could demonstrate that the induction of such tumors, whether in a benign or malignant stage, in otherwise appropriate, comparable, and well-controlled experimental conditions, provides any different kind of evidence for carcinogenesis than the induction of other tumor types. In the evaluation of tumor incidence, therefore, neoplasms in different stages of progression are counted together.

7) *General evaluation of neoplastic pathology for carcinogenesis bioassays* includes consideration of the total number of animals with tumors in each group, the total number of individual tumors, and the index of tumor multiplicity in tumor-bearing animals. The tumor response can be further characterized by a detailed observation of the tumor morphology and related preneoplastic changes. The extent of tumor growth and spread and special morphologic characteristics may give useful indications of the time of development of the neoplastic response. The quality of the pathologic response is determined by a comprehensive evaluation of all the pathologic changes observed in both treated and control animals. Special attention is required in the evaluation of toxic effects other than carcinogenicity, because their pathologic manifestations have to be distinguished from those due to the neoplastic response.

The organs and tissues that are the targets of carcinogens may vary greatly in different species and even under different exposure conditions; therefore, no direct analogy of morphologic response can be expected from a carcinogen in animals of different species and in humans. Examples are known both of widely different target sites [e.g., benzidine induces bladder carcinoma in humans and cholangiomas and liver cell carcinomas in hamsters and rats (*87*)] and of similar responses [e.g., vinyl chloride induces the same type of angiosarcomas of the liver in humans, rats, and mice (*88*)].

Special conditions of tissue exposure or reaction may result in a tumor response by mechanisms that appear due to physical rather than chemical properties of the test material. The following conditions are evaluated differently in this respect:

a) The induction of sarcomas around a "solid state" implant of the test substance into a connective tissue is not considered an indication of the carcinogenicity of that substance when it is administered in another physical form (*75, 76*).

b) The induction of a carcinogenic response by asbestos and other fibrous materials by a mechanism linked to certain physical characteristics such as fiber length and diameter is recognized as a basis for categorizing the exposure to such fibrous materials as a carcinogenic hazard (*22*).

c) The effect of particulate materials in the induction of respiratory neoplasms, when they are administered jointly with certain carcinogens (probably through their capacity to absorb and retain carcinogens, to penetrate the respiratory tract tissues, and to stimulate early cellular responses) is not recognized as evidence of carcinogenicity of these substances but rather as an indication of their role as cofactors in carcinogensis, particulate materials require careful but separate consideration as a potential hazard (*89, 90*).

d) The induction of a neoplastic response by a substance because of its radioactivity is recognized as a cancer hazard.

Other factors are sometimes suggested to be sufficient to refute the presumption of positive evidence of a carcinogenic effect. These factors must be critically examined to avoid false-negative judgments based on unsubstantiated hypothetical explanations of the circumstances of tumor induction. The following factors are considered in this respect:

a) Indirect mechanisms of action requiring special exposure levels or conditions. An example has been suggested in the case of substances that may induce bladder neoplasms only in the presence of bladder stones resulting from high levels of intake and urinary excretion of the test substance (*91*). Support for such a mechanism as an explanation for development of bladder tumors is provided by determination of a specific association of tumors with stones, a dose-response correlation between stones and tumors, and the absence of other chemical or biological indications that the substance might be carcinogenic by other mechanisms. In evaluation of the relevance of such experimental observations to the assessment of human hazard, special consideration is needed for mechanisms by which exposures or intercurrent diseases in the human may act as the cofactor (e.g., in bladder stone induction), thus producing a susceptible state for the possible carcinogenic activity of the test substance.

b) The action of promoting agents only on tissues previously initiated by carcinogens (*51, 52*). Few examples are well documented, such as the phorbol esters in epidermal carcinogenesis in mice. Criteria of risk evaluation need to be defined and dose-response relationships considered. Any claim that a substance acts only by this mechanism and thus is of less concern to humans needs to be supported by experiments showing the mechanism of action and demonstrating that the effect does not occur at human exposure levels.

c) Metabolic pathways of carcinogen activation (*92*) which are suggested as occurring exclusively under certain test conditions in experimental animals but not under other test conditions or in other species. This situation would be important if thorough studies demonstrate that the metabolic pathways for carcinogenic activation of a substance in animals do not occur in humans. Another important situation would be the demonstration that the metabolic pathways of activation of a particular carcinogen identified by studies at high levels of exposure are exclusively formed at such high levels but are absent at lower dose levels.

Statistical Analysis of Results

Statistical hypothesis testing provides an estimate of the likelihood that an experimental observation may or may not be a result of chance alone. The 95% confidence level is widely accepted as a reasonable assurance that the observed effect is real, but confidence that an increased incidence of tumors is a true indication of the carcinogenicity of a substance increases with increasing statistical significance of the results. Thus the level of statistical significance should be reported rather than the fact that a result is statistically significant or not significant at a single preassigned level of confidence. Failure to detect an increase of tumors in a bioassay may be due to an insufficient number of animals tested and does not unequivocally prove that a substance does not pose a risk of cancer.

Tumors rarely seen in experimental animals may raise considerable suspicion even if the statistical significance is well below the 95% confidence level.

Because of the frequent use in chronic studies of both sexes, more than one species or strain, and more than one dosage level, and because many different tissues are examined, a large

number of statistical comparisons are possible between control and treated animals. Thus the results from a chronic study must be interpreted cautiously to control the rate of false positives arising from the large number of possible statistical comparisons (*93*).

Lifetime animal experiments are often difficult to interpret because of competing causes of death, which may alter the pattern of the observation period of the tumor type under study. A common but inadequate form of presenting tumor data is a report only of the proportion of animals in which particular tumor types were observed during the study. This proportion may contain a mixture of three types of observations: 1) The tumor causes the death of an animal and is subsequently observed upon necropsy; 2) an animal dies due to some cause other than a particular tumor and the tumor is observed upon necropsy; or 3) the tumor is observed when an animal is necropsied at the time of a scheduled sacrifice, generally at the termination of an experiment. Simply combining tumors observed under these three situations makes interpretation difficult, and in fact the data may be misleading if the mortality pattern is altered by the toxicity of the substance.

Serial or terminal sacrifices provide an opportunity to compare the prevalence of tumors in various groups of animals unperturbed by mortality. However, sacrifice data do not provide an opportunity to study the effect of a substance on survival or on causes of death.

The analysis of a bioassay is limited by the quantity and quality of data. Such studies must include the age of each animal at the beginning of the experiment, its age at time of removal from the experiment, reason for removal (death, moribund condition, scheduled sacrifice, or others), and all clinical and pathologic observations, including gross and microscopic examination.

When survival curves of control and treated animals differ due to competing causes of death, adjustment of the number of animals at risk may be necessary. For a tumor type generally leading to the death of an animal, statistical analyses of survival experiments should incorporate life-table or competing risk techniques in order to estimate and test tumor incidence. This approach requires assumptions concerning the independence of the competing causes of death. If all the animals are utilized from a survival study, including sacrificed animals, the net probability of death due to a tumor type can be estimated as though that were the only cause of death of a group of animals. Statistical tests for differences between control and treated groups can be performed on the adjusted tumor incidence rates (*94–96*).

For a tumor type that is unlikely to kill the animals, methods of analysis based on life-table techniques are not appropriate for adjusting the number of animals at risk. These tumors are observed conditionally as a result of other events occurring first: death of the animal or a scheduled sacrifice. To estimate the prevalence rate of these tumors, mortality is assumed to be unrelated to the presence of the tumor. Statistical methods for the analysis of tumors that are not generally life-threatening are discussed by Hoel and Walburg (*94*) and Peto (*95*).

SHORT-TERM TESTS FOR CARCINOGENS

Carcinogenesis tests have traditionally been based on the experimental induction of tumors in laboratory animals. Such tests usually involve the observation of treated animals for most of their life-spans.

Recently, short-term methods have been developed to provide more rapid markers for the tentative identification of carcinogenic effects. These methods are directed toward the study of mechanisms underlying neoplastic transformation as well as toward provision of reproducible and rapid methods for testing chemicals and physical agents for potential carcinogenic activity. The use of short-term methods for the evaluation of carcinogens was the subject of a recent review (*97*) from which the following discussion is largely derived.

Methods Based on Genetic Alterations

The analysis of mutagenic effects has been developed mainly to assess the ability of a substance to induce genetic alterations. The resulting information can be used for estimating the genetic hazard of chemical agents for man.

Because of the similarities of basic molecular mechanisms by which chemical mutagens and most chemical carcinogens appear to induce genetic effects (i.e., molecular alterations of DNA), it has been postulated that mutagenic effects can be used to predict carcinogenicity.

The use of a battery of short-term genetic tests is usually recommended in order to minimize false-negative and false-positive results and to select compounds that require further long-term investigations. This battery of tests may include:

a) tests for mutations in bacteria and eukaryotic microorganisms;

b) tests for mutations in somatic mammalian cells;

c) tests for effects on chromosomes in higher eukaryotes, including mammals;

d) evaluation of DNA repair synthesis.

For screening purposes, preference has usually been given to tests that have already been validated with a large sample of compounds belonging to different chemical groups.

Among the mutagenicity tests on microorganisms, the one most widely used and validated is the Ames reversion test in *Salmonella*. Tests in *Escherichia coli, Saccharomyces. Neurospora,* and *Aspergillus* are also being used. Mutagenicity testing is also being conducted in *Drosophila.*

Several other methods currently being evaluated may be used to monitor genetic damage in mammalian cells by carcinogens in vivo and in vitro. These methods include the production of sister chromatid exchanges as well as measurement of the induction of direct damage to DNA and its subsequent repair.

Various short-term mutagenesis tests, some of which are used to provide supportive evidence of carcinogenicity, are discussed in (*98*).

Methods Based on Neoplastic Cell Transformation

Several systems are now available at the mammalian cell level for the identification and study of substances that represent a possible cancer hazard (*99*).

In recent years a number of systems have been developed to test for neoplastic cell transformation by chemical and physical carcinogenic agents. Some of these systems are being used in several laboratories with good reproducibility; other systems are still being developed. Those that have been most widely studied are *a*) the golden hamster embryo cell system and *b*) the mouse embryo fibroblast cell line systems.

In the golden hamster embryo cell system, primary and/or secondary cultures of normal embryo cells are used. Transformation is determined 7–10 days after treatment of cells seeded for colony formation. Quantitation is based on the frequency of morphologically altered colonies.

In the mouse embyo fibroblast systems, established homogeneous cell lines are used. Thus cloned populations of cells can be grown in large quantities and used by many laboratories. Transformants are identifiable 4–6

weeks after exposure to the carcinogen. They may be scored quantitatively by morphologic criteria (focus assay), which correlate highly with tumorigenicity in animals. Among these established lines, the C3H 10T½ Clone 8 cell system has been the most widely studied.

In these tests for neoplastic transformation, the cells derived from transformed colonies or foci, when inoculated into syngeneic or immunosuppressed animals, can grow as malignant tumors. Although the definitive evidence for neoplastic transformation of cells in culture remains their tumorigenicity in animals, a number of phenotypic changes of the cultured target cells are commonly used as indicators.

Other in vitro systems are being developed with the use of specialized cell types such as epithelial cells from liver, epidermis, and other organs. Neoplastic transformation of well-defined epithelial cells by chemicals has been achieved in vitro; conditions for quantitative studies are under development. Such systems may be needed to identify critical target cell populations within target tissues closely correlated with carcinogenesis in vivo.

To be effective, most chemical carcinogens require metabolic activation by cell enzymes to an ultimate reactive metabolite. In mammals metabolic activation of carcinogens takes place in many organs and tissues. Cells in culture can retain enzyme activities, but specific culture systems or preparations may lack or lose the enzyme activity necessary to activate certain chemicals. Therefore, adequate consideration should be given to the effectiveness of metabolic activation functions in each test system used.

Evaluation of Short-Term Test Results

The study of carcinogenesis at the cell level presently offers an effective means to identify carcinogenic effects and mechanisms. In vitro mammalian cell transformation systems are simple models for the study of the mechanisms of chemical and physical carcinogenesis.

As these systems become more widely used as test methods, they will lead not only to better development and definition of screening techniques but also to better understanding of the underlying mechanisms of carcinogenesis.

Short-term tests for chemical carcinogens presently do not, in the absence of animal bioassays and epidemiology data, constitute definitive evidence that a substance does (or does not) pose a carcinogenic hazard to humans. However, positive responses in these tests are considered suggestive evidence of a carcinogenic hazard.

Such positive results also supply supporting evidence to positive animal bioassays or epidemiology results. In some instances results from short-term tests may conflict with animal bioassay data. If an animal bioassay shows a positive response, it cannot be dismissed because a negative response was observed in these tests. However, positive responses in such short-term tests are ordinarily sufficient to provide suggestive evidence of carcinogenicity, even if the substance tested has shown only negative responses in some animal bioassays. As the degree of certainty attached to the negative responses in animal bioassays increases because the observation is reproduced in other animal species and strains or under more rigorous test conditions, the suspicion about the chemical as a result of short-term tests may be reduced and eventually eliminated. These conclusions are in accord with those of the National Cancer Advisory Board's Subcommittee on Environmental Carcinogenesis (*57*):

> At the present, none of the short-term tests can be used to establish whether a compound will or will not be carcinogenic in humans or experimental animals. Positive results obtained in these systems suggest extensive testing of the agent in long-term animal bioassays, particularly if there are other reasons for testing. Negative results in a short-term test, however, do not establish the safety of the agent.

MOLECULAR STRUCTURE AS SUPPORTING EVIDENCE IN IDENTIFICATION OF CARCINOGENS

Information useful in identifying possible carcinogens is provided by their molecular structures. It is well established that certain groupings of atoms (functional groups) in some molecules may impart carcinogenic properties—e.g., some polynuclear aromatic systems, hydrazine groups, N-nitroso groups, and α,β-unsaturated lactones. There is a moderately substantial base of empirical data that permits conclusions about carcinogenic potential on the basis of molecular structure (*33, 100*).

Similarly, some functional groups have never been shown to impart carcinogenic properties to molecules, although the data base for such negative correlations is much smaller and probably inconsequential. The reason for the absence of a strong empirical data base for noncarcinogens is that structure has frequently been used as a guide to testing chemicals for carcinogenicity, and priorities for testing have often been based on the suspected cancer-inducing properties of chemicals.

In some instances, the predictive power of molecular structure of functional groups known to be correlated with carcinogenic properties has proved unsatisfactory. Therefore, the general consensus of the scientific community appears to be that chemical structure has limited value in identifying carcinogens and is to be used in carcinogenesis hazard assessment only as corroborative supporting evidence.

In the absence of other data, however, there are instances in which structure may provide suggestive evidence that a risk of carcinogenesis exists. When strucure is to be used as suggestive evidence, well-documented support should be presented and qualified where necessary by complete notation of substances of similar structure that have been adequately studied for carcinogenic activity.

QUALITATIVE JUDGMENTAL FACTORS IN EVALUATION OF TOTAL EVIDENCE

Evidence that a substance poses a carcinogenic hazard is contributed by each source discussed in the preceding sections of this report: epidemiologic studies, studies on experimental animals, and studies based on short-term and other tests that have been shown to correlate with carcinogenicity; this includes studies of biochemical pathways and chemical structure. For some substances data may be available from all three sources; for others, there may be data from only one or two sources. Each source of relevant data needs to be critically evaluated by consideration of the many aspects discussed in this document.

The judgment that a substance poses a carcinogenic hazard derives from the evaluation of the total evidence provided by all of the sources. Different data sources may not contribute equally to the cumulative evaluation, depending on the specific nature and extent of the data, the scientific quality of the studies, and the adequacy of their documentation.

Conclusions on the carcinogenicity of a substance may be reached on the basis of evidence provided by epidemiologic studies, bioassays in animals, or both. Suggestive evidence is provided by the other types of studies.

In the absence of adequate epidemiologic or animal evidence, a positive response in any of the short-term in-vitro tests that correlate with carcinogenicity is considered suggestive of a carcinogenic hazard. Suggestive evidence may also derive from

considerations of chemical structure or biochemical pathways.

Ordinarily, if a substance has produced positive results in a single adequately designed and conducted animal bioassay and no other data are available, the conclusion is that the substance is likely to pose a risk of cancer to humans. These results may be further confirmed by data on chemical structure, in vitro testing, or relevant biochemical studies that suggest a carcinogenic potential. However, negative data from the latter three sources do not override adequate positive data from an animal bioassay. Further confirmation that the substance poses a carcinogenic hazard to humans is obtained from bioassay data showing reproducibility of results, positive dose-response relationships, and concordance of results (*see* "Evaluation of Pathologic Results").

Because of biologic variability among species, the conclusion that the evidence is positive on the basis of results obtained in one animal species is not altered by negative data obtained in other species or strains of test animals. Moreover, negative epidemiologic data, questionable because of limitations in the power of detection of such studies, do not deny the conclusion of carcinogenicity on the basis of animal bioassays. Negative evidence from properly designed and conducted epidemiologic studies may, however, be used to set an upper limit on human risk to comparable populations under analogous conditions of exposure.

It should be stressed that the qualitative judgment whether a substance poses a carcinogenic hazard is based on the evaluation of cumulative evidence from all pertinent data sources. The reasons for specific conclusions need to be clearly detailed.

The terms "strong" and "weak" have been used in the literature to describe both the nature of the hazard or risk and the exent and quality of the evidence. A certain confusion may have ensued, since one could refer to weak evidence of a strong effect or to strong evidence of a weak effect. The two categories are clearly not equivalent and should not be confused.

PART III. THE QUANTITATIVE ESTIMATION OF RISK

The previous section of this document dealt with the issue of the likelihood that a substance poses a carcinogenic hazard to humans. In some instances a regulatory agency may be required, or may find it useful, to estimate quantitatively the cancer risk of such a substance in exposed humans if the compound is assumed to be a human carcinogen.

Quantitative assessment of human cancer risk may be based on epidemiologic or animal data. In either instance, methodologic problems arise because of the need to extrapolate from effects observed under one condition and level of exposure and in one population group or biologic system to arrive at an estimate of the effects expected in the human group or individual. Because extrapolations are involved, uncertainties are necessarily attached to the cancer risk estimates that can be made with current methodologies. Furthermore, uncertainties arise from other sources, particularly from attempts to identify accurately conditions and levels of exposure of the human group or individual.

Despite the uncertainties, risk estimates can be and are being made, not only by some regulatory agencies but by other scientific bodies. Because of the uncertainties, however, and because of the serious public health consequences if the estimated risk were understated, it has become common practice to make cautious and prudent assumptions wherever they are needed to conduct a risk assessment. This approach has a precedent in other areas of public health protection where similar problems arise because of gaps in knowledge (*101, 102*). Thus current methodologies, which permit only crude estimates of human risk, are designed to avoid understatement of the risk. It must be recognized, however, that in some circumstances this cannot be guaranteed because of other factors that may enhance human response, such as synergistic effects. Thus risk assessments should be used with caution in the regulatory process.

If data on animals are used as the basis for estimating human risk, data obtained from the most sensitive animal species or strain tested are commonly recommended as the starting point for extrapolation. Of the available data, these are clearly the least likely to understate human risk. Use of data from less sensitive species or strains is justifiable only if there are strong reasons to believe that the most sensitive animal model is completely irrelevant to any segment of the exposed human population.

A limited comparison of human and animal data for carcinogens is contained in a report of the National Academy of Sciences (*103*). Data were compared for benzidine, chlornaphazine, diethylstilbestrol, aflatoxin B_1, vinyl chloride, and cigarette smoke. The authors stated that "* * * as a working hypothesis, in the absence of countervailing evidence for the specific agent in question, it appears reasonable to assume that the lifetime cancer incidence induced by chronic exposure in man can be approximated by the lifetime incidence induced by similar exposure in laboratory animals at the same total dose per body weight." These preliminary observations suggest that current methodologies may not lead to serious errors.

Whether quantitative risk assessment is based on data from animals or humans, there is uncertainty about the shape of the dose-response relationship at the (usually low) levels of actual human exposure. Mathematical extrapolation models are discussed in detail later in this section. The linear nonthreshold dose-response model is most commonly used at the present time. Of the various models, it appears to have the soundest scientific basis and is less likely to understate risk than other plausible models. It has, for many of the same reasons, a long history of use in protection against radiation (*101, 102*).

The most favorable foundation for quantitative risk assessment is based on well-characterized responses in human populations with well-defined exposures. Unfortunately, the exposure estimates are often unavailable or crude. Negative epidemiologic studies on populations for which usable exposure estimates are available can be valuable in conjunction with animal data; the studies on animals provide evidence for carcinogenic hazard, and the epidemiologic data may provide upper limits of response for cross-comparison with the animal data. Although extrapolation from the observed human population group to other groups carries less uncertainty than extrapolations from animals to humans, the possibility of significant differences in the characteristics and conditions of exposure of the two population groups must be recognized. Any such differences that may affect the estimate of risk should be noted, although information is rarely available that will permit specific integration of these factors into the risk assessment methodology.

To the extent currently possible, the methods described in the following section permit a crude order-of-magnitude estimate of risk for substances that may pose a cancer hazard to humans. As more knowledge develops, risk assessment methodologies should be improved. Some of the kinds of information and

knowledge that will likely prove useful in the future are discussed in the sections to follow. At present, most such information is not available and thus cannot ordinarily be used in risk assessment without the imposition of numerous assumptions. Caution is needed in risk assessment as long as these gaps in knowledge exist.

Much has been written about threshold doses for carcinogenic effect, but unfortunately, there is no recognized method for determining their existence. A model recently proposed by Cornfield (*104*) permits the inclusion of thresholds. However, as Cornfield stipulated originally and again recently (*105*), a threshold could be derived from this model only if there were instantaneous and complete deactivation of the material before any carcinogenic effect occurs—an improbable event.

Since threshold doses for carcinogenesis have not been established, a prudent approach from a safety standpoint is to assume that any dose may induce or promote carcinogenesis. Some of the mathematical models proposed to describe the dose-response relationship for carcinogenesis are discussed in the following section.

With the present state of knowledge, the quantitative assessment of cancer risks provides only a rough estimate of the magnitude of the cancer risks; this estimate may be useful in setting priorities for control of carcinogens and in obtaining a very rough idea of the magnitude of the public health problem posed by a given carcinogen.

MATHEMATICAL MODELS FOR HIGH-TO-LOW DOSE EXTRAPOLATION WITHIN A SINGLE BIOLOGIC SYSTEM

Mathematical models were developed in the last two decades for estimating the effects of exposure levels well below levels for which test data were available, with the goal of ensuring that the risk will not be underestimated. These models of dose-response relationships make use of data obtained in a given biologic system to extrapolate from high to low doses. Consideration must be given to the many biologic variables that influence the level of response in different species or under different exposure conditions.

The Models

In order to extrapolate outside the experimental range of exposure levels, some mathematical formulation relating response to dose must be available. The two categories of mathematical models commonly used to depict the relationship between response and dose are dichotomous-response models and time-to-response models. In the dichotomous-response situation the response of interest is the presence or absence of some specified condition. Time-to-response models attempt to relate dose level to distribution of the time until the occurrence of a given event, such as tumor observation or death. (Both categories of models are completely specified except for a few unknown parameters, which are typically estimated from a given set of experimental data.)

A variety of different approaches have been proposed to deal with the problem of low-dose extrapolation involving a dichotomous response. Included are the Mantel-Bryan procedure, the one-hit model, linear extrapolation, and various extensions of the multistage model developed by Armitage and Doll (*106*).

Mantel and Bryan (*107, 108*) proposed an extrapolation technique based on the log-probit model, which had long been used for bioassays to estimate median lethal doses. They selected this model because it seemed to provide a reasonable fit to a large body of experimental carcinogenesis data and not because of any mechanistic arguments in its support. Under this procedure, extrapolation is conducted from the upper confidence limit on the observed experimental response along a probit log-dose line with a preassigned slope of one to some specified low level of risk. By using the upper confidence limit and fixing the slope at one (a shallower slope than they had typically seen with their experimental data sets), Mantel and Bryan hoped to generate an upper bound on the estimated dose associated with the predeterminated risk level, regardless of the true form of the underlying and unknown dose-response curve. However, subsequent theoretical and applied research has demonstrated that the Mantel-Bryan procedure is not as conservative as once thought and may underestimate risk in some situations (*109, 110*).

The one-hit model is based on the concept that a tumor can be induced after a single susceptible target or receptor has been exposed to a single effective dose unit of a substance (*109, 110*). Thus, unlike the Mantel-Bryan procedure, there is an assumed biologic mechanism of action for the carcinogen underlying the one-hit model. This action implies that the probability (P) that a tumor will be induced by exposure to a chemical at dose d is given by the equation $P(d)=1-\exp[-\lambda d]$, where λ is an unknown non-negative constant. When λd is small (i.e., in the low-dose region), it can readily be shown that $P(d)\approx\lambda d$, i.e., for low dose levels the one-hit model is well approximated by a simple linear model in which the probability of tumor observation is directly proportional to dose.

If the unknown (true) dose-reponse curve is assumed to have a sigmoidal shape—an assumption supported by a wealth of toxicologic data—then the response will curve upward in the low (or, typically, environmental) dose region. Thus a linear model will provide an upper bound to curves of this shape and, it is hoped, a conservative estimate of the dose associated with any specified level of risk (*111*). A line connecting zero with a point on the dose-response curve for the excess tumor rate above background will always lie above the true dose-response curve for the convex portion of the curve. An additional degree of conservatism is introduced by extrapolating back to zero from an upper confidence limit (UCL) for the net excess tumor rate above the background rate. In the linear model the tumor rate is assumed to be proportional to dose: $P(d)=\lambda d$. The upper confidence limit for the slope λ is UCL÷experimental dose. Thus the maximal risk for a given dose d may be estimated by the equation maximal risk$=(\mathrm{UCL}/d_e)\times d$, where d_e is the experimental dose. Conversely, the equation for a predicted dose for a maximal level of risk is: predicted dose$=(\mathrm{risk}\times d_e)/\mathrm{UCL}$.

A number of investigators have published papers (*112–115*) based on the Armitage and Doll (*116*) formulation of the multistage model of carcinogensis. Under the multistage model it is assumed that the cancer originates as a "malignant" cell, which is initiated by a series of somatic-like mutations occurring in finite steps. It is also assumed that each mutational stage can be depicted as a Poisson process in which the transition rate is approximately linear in dose rate. Then the lifetime probability of tumor induction can be expressed approximately as $P(d)=1-\exp(-\lambda_0-\lambda_1 d-\ldots-\lambda_k d^k)$, where $\lambda_i\geq 0$ for all values of i, and k corresponds to the number of transitions or mutational stages. (Highly sophisticated computer algorithms have been developed for fitting the multistage model to laboratory data with the use of a restricted maximum likelihood approach which does not require that the value of k be pre-specified.)

Both the total incidence of tumors and the time at which tumors occur are important. Tumors leading to early

death and life-shortening need to be considered.

Time-to-tumor is the time at which a tumor is detected or observed by palpation or by gross or microscope examination of an animal at the time of death or sacrifice. Time-to-tumor is not used here to indicate the instant at which a pretumorous condition becomes a tumor. Time-to-observance is better terminology.

Some hope for improving risk estimates has been based on use of the time-to-observance of tumors in addition to use of the proportion of animals possessing tumors. On the basis of Druckrey's work (*117*), the median time to tumors appeared to increase as the dose decreased. It was hoped that low doses could be found that would result in median times-to-tumor observation well beyond the expected lifetime; this might result in the identification of "practical thresholds." Albert and Altshuler (*118*) expanded on the use of median time-to-tumor observance by employing distributions of time-to-tumors for individual animals. Chand and Hoel (*119*) showed that use of a log-normal time-to-tumor distribution leads to a probit-log dose relationship, and use of a Weibull time-to-tumor distribution leads to an extreme value model for the proportion of animals with tumors: $P(d)=1-\exp[-\exp(\alpha+\beta \log d)]$, where alpha and beta are constants. Schneiderman et al. (*120*) demonstrated that even though the median time-to-tumor may be well beyond the expected lifetime, a significant proportion of animals or humans may still develop tumors within the normal life-span. Peto (*121*) examined human data and questioned the concept that lower doses result in longer latency. Whittemore and Altshuler (*122*), analyzing data on cigarette smoking, concluded that it was not possible to distinguish between the log-normal and the Weibull models.

The available data do not permit a conclusion as to whether lower doses lengthen the latency periods. Animal experiments at high doses may induce more tumors resulting in easier and therefore earlier detection, and this may not be due to an actual decrease of latency period.

Time-to-observance response models have not received the same degree of attention as dichotomous-response models in carcinogenesis risk extrapolation. One of the major factors underlying this relative lack of emphasis may be that studies in which animals were given the substance in their feed have not generated sufficient information to determine the relationship between age and cumulative cancer incidence.

Procedures

In the preceding section it was noted that the Mantel-Bryan procedure is essentially empirical and lacks biologic relevance with respect to current knowledge about carcinogenesis. Since risk extrapolations developed by the Mantel-Bryan technique tend to zero much more rapidly in the low-dose region than do extrapolations based on somatic mutation models, the Mantel-Bryan procedure would certainly not be appropriate if the carcinogen under study were thought to act directly on cellular DNA (*109*).

Initially, extrapolation based on a multistage model appears to offer significant advantages over linear extrapolation procedures. Under the multistage approach, no assumptions are made a priori about the exact form for the mathematical extrapolation. Instead, the experimental data are used to estimate the shape of the dose-response curve. However, Crump et al. (*109, 114*) and Guess et al. (*110*) have shown that the upper confidence limit on estimated risk becomes essentially linear for generalized polynomial extrapolation in the low-dose region. This approximate linearity holds even when the maximum likelihood estimate of excess risk does not contain a linear component (estimated). Therefore, there is some question whether the mathematical refinements of generalized polynomial extrapolation are justified for application to animal bioassays, which may be only crude approximations to the human situation (*109*).

As an interim procedure, it has generally been recommended (*106*) that whenever quantitative risk analysis is deemed necessary, *linear extrapolation should always be included* among any methods used unless there is reason to believe that the experimental (observed) response does not fall in the convex portion of the dose-response curve. If the response is in the concave portion of the curve, the one-hit model is suitable. At low observed responses the linear and one-hit models yield nearly identical results. An added degree of protection can be achieved by starting the extrapolation from the upper confidence limit of the response.

The mathematical procedures per se are intended to provide upper limit estimates of risk from a statistical standpoint. However, the risk estimates as applied to humans should not be regarded as upper limit estimates because of large biologic uncertainties (*see* "Extrapolation From Observed Effects to Estimates of Risk for the Observed Population").

CHARACTERIZATION OF POPULATION EXPOSURE

The estimation of total population exposure to a given substance (and/or to its decomposition and metabolic products) requires consideration of the following aspects:

a) sources of human exposure (occurrence, production, uses, and environmental distribution);

b) analytical methods for detecting and measuring exposures in the environment and in the population;

c) routes and conditions of exposure;

d) duration, frequency, and intensity of exposure; and

e) size and characteristics of the exposed populations.

During examination of exposure data, important qualitative and quantitative factors beyond definable numerical values of dose level and population size will emerge; although such information may not be usable directly in a mathematical calculation of risk estimate, it will frequently provide additional perspective and insight during risk evaluation. Because of the great diversity in sources and estimating procedures available in various situations, it does not seem practicable at this point to set minimum detailed specifications for the reliable estimation of exposures or to identify recommended or approved methods and procedures for producing exposure estimates. The following general considerations indicate the kind of data useful for assessment of population exposures. The better defined these data are, the higher will be the confidence that a realistic estimate of risk for the exposed populations has been made (*123*).

Sources of Human Exposure

Two types of exposure sources are considered: primary sources and human contact sources.

Primary sources of exposure to a chemical are those that determine its release into the human environment, and they include natural occurrence, extraction from natural products, mining, chemical synthesis, manufacturer or production, and specific uses.

Human contact sources are those that bring about the contact of the substance with the human body, and they include items, or preparations containing the chemical (such as foodstuffs or consumer products), vehicles, or a medium in which the chemical is present (such as ambient air or drinking water).

Some substances may originate from a single primary source and be present in a wide range of human contact sources; conversely, a specific human contact source may be traced to several

different primary sources. It is important that for each substance the entire range of sources and environmental distribution be examined.

Frequently, there is more than one source of human exposure, and an individual may be exposed to a substance of concern from an array of sources depending upon the circumstances. Analysis of environmental distribution and exposure pathways allows idenification of the most significant sources, so that both the size of the population exposed and the intensity of exposure can be established.

In some instances, it is possible to estimate combined exposures to the same substance from different sources, primarily where the populations affected by these different sources are the same. Frequently, however, differences in the populations exposed from various sources are so large that any attempt to combine the estimates may produce an unrealistic or unclear description of the acutal human exposure conditions. Then it is preferable to consider each source separately and subsequently use whatever knowledge is available on multiple sources of exposures to interpret these observations.

Estimates of the total level of production of a substance can be useful indicators of the extent of exposure, particularly over time. Dates of first synthesis and commercial production of a substance are useful in the evaluation of delayed toxic effects and allow an estimate of the time before which no human exposure could have occurred. The accuracy of data on national production and foreign trade of individual substances (which are often difficult to obtain) needs to be ascertained.

Uses of a substance are important descriptors of its environmental distribution and the extent of human exposure. Whenever possible, all uses of carcinogenic substances should be identified.

An important distinction is that between uses for which human exposure is intended (intentional exposures) and that for which it is not intended (unintentional exposures). Individual exposure or consumption of a substance may be voluntary or involuntary. The sociologic bases and implications of these definitions are beyond the scope of this report.

Analytical Methods for Detection and Measurement of Exposures

The specificity and limit of detection of analytical procedures for the identification of many carcinogenic substances, both in the environment and in exposed organisms, have been remarkably improved in recent years. Progress in analytical chemistry is expected to undergo further refinement and improvement in the near future.

The limit of detection of analytical methods varies considerably for different substances and different conditions of analysis, and this is a critical factor in assessing a source of exposure. It is important to consider that the agent may not be measurable but may still be present below the minimum detectable level. The minimum detectable level of a substance may vary depending on different vehicles, media, and conditions of exposure.

Quantitative determinations of the level of a substance in various exposure sources should consider time and space distribution and variations, and ranges of values may be useful to estimate the conditions of exposure.

The chemical and physical properties of the substance should be identified. Such characteristics as particle size distribution for aerosols and dust should be determined insofar as possible.

Analytical determination of the levels of a substance in exposed organisms, particularly in the exposed population, is of great value but not always obtainable. Available data on the levels of substance (or its metabolites) in the target tissues or body fluids should be considered.

The dose of an ultimate carcinogen at the site of action in the tissues or cells, which is measured at all times after its introduction ("target tissue dose") is ideally the dose that should be estimated and correlated with expected effects. This target tissue dose usually cannot be closely estimated because of many variables and uncertainties (*102*). The relationship between target tissue dose and exposure dose may vary considerably under different conditions. To the extent practicable, documentation of the analytical methods, the sampling conditions, the limits of detectability, and the range of observed values is desirable.

Routes and Conditions of Exposure

All possible routes of exposures associated with each source should be identified. If any routes of exposure are considered irrelevant for estimation of effective doses, the circumstances should be specified. Careful consideration of sources of exposure—e.g., product use patterns, environmental or occupational situations, and background—may suggest or reveal routes of exposure not immediately apparent. For example, a chemical may also be absorbed through the skin or by ingestion when inhalation is apparently the primary route.

For estimation of animal-to-human correlations in the evaluation of test data on animals, it is necessary to obtain the human dose level in units consistent with those used to describe the effective dose in the animal bioassay being used for comparison. In some instances, any necessary conversion from the actual measurement at the source to the needed units describing exposure dose can be straightforward (e.g., by simple application of observed or estimated food ingestion rates to a chemical's concentration in a food). In other instances, complex calculations or modeling procedures may be necessary, as in the estimation of effective exposure distributions from ambient air on the basis of monitoring data or emission inventories for point sources. This conversion or translation step, often necessary in the estimation of human exposure, should always be explicity identified and reported. When available data show substantial differences between the route and conditions of exposure in test animals and in humans, it is necessary to rely on estimates of comparability and to attempt to establish an acceptable equivalent dose. In the absence of satisfactory equivalent dose data, only defensible conservative assumptions should be used in such a way that the possible risk is not underestimated.

Duration, Frequency, and Intensity of Exposure

An important factor in the quantitative evaluation of population exposure is the length of time during which exposures occur. Although the time of exposure may vary considerably within a population, there are cases when it can be reasonably well-defined. These include cases of specified duration of exposure (e.g., to certain drugs or certain occupational carcinogens) or continuous lifetime exposure to widely disseminated environmental carcinogens (e.g., polycyclic aromatic hydrocarbons).

Effective exposure rates corresponding to typical patterns of individual exposure, whether short-term or long-term temporal trends, must be reported wherever significantly different patterns exist. The two components of the estimated level or amount of exposure—the effective rate per unit time or per incident of exposure and the frequency-duration pattern—should be explicity identified for each exposure pattern considered.

Size and Characteristics of Exposed Populations

The total number of people exposed to any level of a carcinogenic substance represents a major indicator of the extent of risk related to that substance. Because combinations of exposures to different carcinogens may contribute to the cancer risk in the same population or individual, and because no threshold level for exposure to a carcinogen can presently be reliably determined for a population, a contributory risk level from any exposure level, however small, must be assumed.

Age of exposure should be considered, i.e., whether exposure is essentially lifelong (at more or less constant rates) or is concentrated in certain age ranges. The relationship between total lifetime exposure in each exposure pattern and the amount of this exposure that may be concentrated in any specific age ranges should be identified. Wherever feasible, the degree of stratification of exposed populations should be identified to permit distinctions between effective exposure amounts by age (e.g., childhood, working age, and elderly age groups) and by sex. As noted above, populations having high-risk age groups should be identified. Attention should be given to exceptional exposure groups of special concern, such as infants, children, and pregnant women, as well as to groups with special genetic conditions or concurrent disease. In addition, in descriptions of certain population subgroups, the smoking habits, dietary and alcohol consumption patterns, and other cultural and environmental characteristics should be considered if possible.

EXTRAPOLATION FROM OBSERVED EFFECTS TO ESTIMATES OF RISKS FOR EXPOSED POPULATION

The quantitative estimation of risk from a carcinogenic substance for the entire exposed or potentially exposed population may be conducted with the use of observations on the effects of the substance in 1) a defined human population group and 2) experimental animal tests.

In both situations the extrapolation will take into account the factors that characterize and distinguish the groups observed and the factors to which the extrapolation applies.

Correlations From Observed Human Population Groups to Others

The problem to be considered here is the estimation of present or potential risks for all people exposed to a given substance by means of data obtained from observations in a defined population group. The observed group may be small and its exposure conditions may be well defined, as for certain studies of drugs or for occupational exposures. In other situations the observed group may be poorly defined, even if larger. In analyzing the correlation between observed and estimated population effects, it is desirable where feasible to review the critical differences between the two conditions, such as age and sex distribution of the population; genetic, racial, and ethnic differences; environmental differences and migration patterns; dietary and cultural habits; smoking patterns; alcohol consumption; patterns of intercurrent disease; and particular susceptibility states including pregnancy and fetal and neonatal exposures. Many of these complex variables are considered under "Epidemiologic Evidence" in Part II and "Characterization of Population Exposure" in Part III.

Animal-to-Human Correlations

Although a close qualitative similarity has been established in the nature of the response of laboratory animals and humans to carcinogenic substances, a quantitative correlation is more uncertain because of the marked variation of susceptibility in different animal species and among individuals in the human population. It is not possible to reduce the variables to a single safety factor for general use (*106*).

Several species-conversion factors should be considered in estimating risk levels for humans from data obtained in another species. Species–conversion factors are affected by many variables, such as body surface, body weight, metabolic pathways, nutritional conditions, genetic variability, and bacterial flora as well as tissue distribution and the retention and fate of the chemical. In evaluating exposures to the general population, one should consider all ages, transplacental exposures, concurrent disease conditions, and special susceptibility states.

Other conversion factors should also be considered when observations are obtained for test species under exposure conditions markedly different from those in the population (e.g., different routes or modes of exposures, vehicles, modifying factors, variations in age, sex, perinatal exposures, disease states, and single vs. multiple exposures). The limits of uncertainty should be stated whenever possible (*102, 106*).

Different carcinogens tested under comparable experimental conditions show a wide range of response; if extreme cases are included, the range of variation is more than one millionfold. Changes in experimental conditions, particularly ones that alter the effective dose, can markedly affect the observed level of effect of a carcinogen within the same genetic strain of animal. Exposure of experimental animals to certain other chemicals in addition to a carcinogen under test may change the observed effect in either direction and at the extremes up to one hundredfold or even one thousandfold (*124*). Differences between species can be even greater. On the other side of the correlation, the human response to carcinogens as well as to many other chemicals and drugs may also show great quantitative variations among individuals. Studies on the metabolic activation and chemical interaction of carcinogens in human tissues in vitro have shown interindividual quantitative variations of about one hundredfold in relatively small population samples (*125–127*). Individuals resistant or sensitive to one carcinogen may not be equally resistant or sensitive to another carcinogen or to combined effects of several exposures. Such wide interindividual variations are also well known from many pharmacokinetic studies.

A number of variables are relevant to the correlation of animal and human conditions. Some problems inherent in the use of animals must be kept in mind when animal studies are used for estimation of the quantitative carcinogenic potential of a substance for humans. A concise statement of some of these factors is contained in "Drinking Water and Health," prepared by the Safe Drinking Water Committee, Advisory Center on Toxicology, National Research Council, National Academy of Sciences (*56*). Factors discussed in this document include the rate of chemical absorption, distribution within the body, metabolic differences among exposed animals, effect of intestinal bacteria, rates of excretion and reabsorption, differences in molecular receptor sites for the carcinogen, environmental and genetic differences, and number of exposed animals and susceptible cells.

Metabolism and pharmacokinetics account for major differences in sensitivity to chemical carcinogens between species. In principle, this information could be used in estimating the relative sensitivity of humans compared to experimental animals. In practice, detailed metabolic pathways in humans are not known for many carcinogens; moreover, the marked variation in metabolism and sensitivity

among individuals of different ages, states of health, and other biologic conditions require more information on the heterogeneity of human metabolic and pharmacokinetic responses than is usually available. It is hoped that future research will clarify these important correlations in much greater depth. Such information, if available, should be used to correct for an underestimate of human risk, but it should be used to correct for an overestimate of human risk only when there is substantial information on diversity of human response.

The contribution of animal test data to the estimation of the risk level for humans should be based on experiments with the most sensitive species available. Confidence that this procedure will not underestimate the human risk increases with the number of experiments and the number of species and strains studied.

LACK OF PREDICTABLE THRESHOLDS FOR AN EXPOSED POPULATION

The self-replicating nature of cancer, the multiplicity of causative factors to which individuals can be exposed, the additive and possibly synergistic combination of effects, and the wide range of individual susceptibilities work together in making it currently unreliable to predict a threshold below which human population exposure to a carcinogen has no effect on cancer risk.

Observation of the marked individual differences in the response of human subjects to carcinogens shows that some individuals do not develop cancer in their lifetime, whereas others develop it readily after the same exposure to a carcinogen. Although these observations are compatible with the existence of different "thresholds" for individual subjects in certain conditions, they are not a basis for predicting a no-effect level of a carcinogen in other individuals or under different conditions. There is no presently acceptable way to determine reliably a threshold for a carcinogen for an entire population.

Individual human subjects in the population are exposed throughout life to a number of carcinogens, which may be considered to provide a background of carcinogenic risk; exposure to any amount of a single carcinogen, however small, is regarded as capable of adding to the total carcinogenic risk(*109*). Cancer susceptibility varies greatly among individual members of human populations due to genetic, racial, and ethnic factors; to environmental and dietary exposure; and to other modifiers.

Variability among individuals makes it very difficult to have confidence that an observed no-effect level of exposure in animals or even in a specific human population (for which individual variation may be small in comparison to the total population) will be applicable to the total human population at risk. A large number of factors (e.g., age, sex, race, nutritional status, immunologic status, general state of health, previous exposure to the substance in question or to other substances) could affect individual susceptibility. Even if thresholds for carcinogens could be demonstrated for certain individuals or for a defined population, no reliable method is known for establishing a threshold that could apply to the total human population (*67*).

SUMMARY OF RISK ESTIMATION

For a given substance, the usefulness of dose-response data obtained from a specific human population group or from animal tests for estimation of risk in the general population is limited by the consideration that general population exposures to one substance are usually only a component of the total carcinogenic burden derived from multiple sources, with their possible interactions.

Recognition of these limitations, however, does not imply that no attempt should be made to develop reasonable risk estimates for different conditions of human exposure. The several components of quantitative risk assessment include the following:

a) definition and quantification of exposures;

b) characterization of the exposed populations in quantitative terms;

c) chemical and physical properties of the substance and its chemical reactivity in relation to exposure;

d) prudent quantitative mathematical extrapolation of the responses from observed to estimated exposure ranges within the observed biologic system; and

e) qualification of the estimated risk in light of identifiable biologic and toxicologic differences that may be present in the exposed human population.

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